Tug of War in Spindle Orientation

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The evolutionarily conserved Gαi-LGN-NuMA protein complex is a key regulator of spindle orientation, but how its spatiotemporal localization is achieved remains elusive. Chiu et al. (2016) show that SAPCD2 negatively regulates LGN cortical localization by competing with NuMA for LGN binding in both epithelial cells and mouse retinal progenitor cells.

Oriented cell division is essential for the development of stem cell lineages and for tissue homeostasis. During asymmetric cell division, correct division orientation depends on proper spindle orientation. In the developing vertebrate retina, while the majority of retinal progenitor cells (RPCs) divide horizontally within the plane of the neuroepithelium, a small fraction of RPCs at late stages of retinogenesis reorient their mitotic spindle to divide vertically at perpendicular angles (Cayouette et al., 2001). The highly conserved ternary complex Gαi-LGN-NuMA and the minus-end-directed Dynein that regulate spindle orientation in various organisms and developmental contexts also control vertical division of RPCs (Gotta et al., 2003; Konno et al., 2008; Kotak et al., 2012; Schaefer et al., 2000; Yu et al., 2000). Gαi-GDP, tethered at the plasma membrane, interacts with the C-terminal GoLoco motifs of LGN to recruit LGN to the cell cortex. During mitosis, LGN interacts through its N-terminal Tetratricopeptide repeats (TPRs) with the microtubule-binding protein NuMA (Du and Macara, 2004). The simultaneous binding of Gαi and NuMA to LGN results in a conformational switch of LGN, from a "closed" state to an "open" state, by disrupting the intramolecular association of N and C termini of LGN (Du and Macara, 2004). This ternary complex anchors Dynein at the plasma membrane to ensure proper spindle positioning (Kotak et al., 2012). However, the delicate spatiotemporal regulation of Gαi-LGN-NuMA localization is poorly understood. Specifically, it was unclear how the exclusion of the ternary complex from the apical domain occurs in RPCs.

In this issue of *Developmental Cell*, Chiu et al. (2016) now show that Suppressor APC domain containing 2 (SAPCD2) is a binding partner of LGN and a negative regulator of Gαi-LGN-NuMA localization, both in RPCs in vivo and epithelial cells in vitro. To identify new regulators of the Gαi-LGN-NuMA ternary complex, Chiu et al. performed protein purification and mass spectrometry using a FLAG-tagged GTPase-deficient Gαi-Q204L and uncovered SAPCD2 as a potential Gαi interactor. Their subsequent analyses demonstrated that this interaction is likely indirect and bridged through direct binding between SAPCD2 and AGS3/LGN. Remarkably, SAPCD2 interacts with the N-terminal TPR motifs of LGN, a region that is known to bind to NuMA. This raised the interesting possibility that SAPCD2 and NuMA may compete for the binding to LGN. Indeed, the authors convincingly demonstrate that NuMA inhibits the association of SAPCD2 and LGN in a dose-dependent manner. However, the reverse-competition assay remains to be carried out to establish that SAPCD2 is capable of inhibiting the association between NuMA and LGN. Next, the authors show that in mouse retina, SAPCD2 is expressed in RPCs and newly differentiated neurons, but not in mature retinal cells. Intriguingly, SAPCD2 predominantly localizes to the apical pole in horizontally dividing RPCs and is excluded from the lateral membrane where LGN and NuMA are observed. In contrast, SAPCD2 is hardly detectable in vertically dividing RPCs, in which LGN is expressed throughout the cell cortex. These observations collectively indicate a complementary expression pattern between SAPCD2 and LGN in RPCs, supporting the hypothesis that SAPCD2 is able to compete with NuMA for LGN binding.

To ascertain the role of SAPCD2 in spindle orientation, the authors first studied its function in organotypic 3D-cultured MDCK cell system, in which the Gαi-LGN-NuMA ternary complex is known to regulate spindle orientation. SAPCD2 is also apically localized in polarized MDCK cell monolayers. Importantly, they demonstrate that knocking down *SAPCD2* randomizes mitotic spindle orientations, leading to cystogenesis defects. Remarkably, the authors also show that loss of *SAPCD2* leads to a 6-fold increase of vertical divisions in mouse RPCs, strongly supporting a role for SAPCD2 in regulating spindle orientation. Prior studies have established a correlation between spindle orientation and symmetric/asymmetric division output in mouse retina (Cayouette and Raff, 2003). To trace the terminal divisions of RPCs, the authors perform retroviral lineage tracing and identify cell fates in two-cell clones. In control animals, the vast majority of RPCs undergo symmetric terminal divisions to produce two photoreceptor cells. In *Sapcd2*$^{-/-}$ retina clones or clones with short hairpin RNA (shRNA) knockdown of *Sapcd2* in RPCs, significantly increased population of RPCs undergo asymmetric terminal divisions, producing a photoreceptor cell and a bipolar, amacrine, or Müller glial cell. Therefore, Chiu et al. (2016) demonstrate a direct causal link between spindle orientation and cell fate determination in RPC terminal divisions.

How does SAPCD2 regulate spindle orientation? Given that SAPCD2 potentially competes with NuMA for LGN

binding, and given the complementary expression pattern between SAPCD2 and LGN in RPCs, the authors hypothesize that SAPCD2 regulates spindle orientation by interfering with LGN localization. To test this model, the authors overexpressed SAPCD2 in HeLa cells and found that this effectively reduces the abundance of LGN cortical localization in these mitotic cells. Conversely, depletion of SAPCD2 dramatically enhances the cortical localization of LGN. Moreover, the enhancement of LGN cortical localization by Gαi overexpression was suppressed by SAPCD2 overexpression. Remarkably, in *Sapcd2*$^{-/-}$ mutant retina, apical localization of LGN in RPCs increased almost 3-fold compared to the control. All of these observations indicate that SAPCD2 regulates spindle orientation by interfering with the cortical localization of LGN. Future work is needed to test whether SAPCD2 function ultimately influences Dynein localization in RPCs.

What anchors SAPCD2 at the apical cortex in horizontally dividing RPCs is still unclear. Tight junction protein PATJ, which may also interact with SAPCD2 (Chiu et al., 2016), may tether SAPCD2 to the cell cortex. It remains to be tested whether PAR3, identified in the same SAPCD2 immunoprecipitation experiment, provides the asymmetric cue for SPACD2 in RPCs. In addition, it will be of great interest to identify the mechanisms that control the differential localization of SAPCD2 in horizontally and vertically dividing RPCs. Another key regulator and interactor of LGN is Inscuteable (Insc), which is functionally conserved in flies and mammals (Schaefer et al., 2000; Yu et al., 2000; Zigman et al., 2005). Mammalian Insc (mInsc) localizes to the apical side of vertically dividing RPCs and is concentrated at poles and apical cortex in horizontally dividing RPCs (Zigman et al., 2005). Given that Insc and SAPCD2 positively and negatively regulate LGN localization, respectively, future work will be required to determine whether mInsc and SAPCD2 compete for apical localization in RPCs to regulate spindle orientation, ultimately deciding horizontal versus vertical divisions of RPCs.

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Cybernetics, Redux: An Outside-In Strategy for Unraveling Cellular Function

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A new paper in *Science* reveals how repetitive stimulation can identify and help to repair fragilities within a signaling network, while using linear mathematical models inspired by engineering, thereby suggesting how cybernetic methods can be integrated into systems and synthetic biology.

Wendell Lim's laboratory at UCSF has published a report in *Science* in which repetitive pulsing of *Saccharomyces cerevisiae* cells with an osmotic shock revealed what the authors—Amir Mitchell, Ping Wei, and Wendell Lim—described as an "Achilles' heel" in the MAP kinase signaling network (Mitchell et al., 2015). This work builds upon a long tradition of exploiting engineering ideas in biology but also suggests how such methods can be more effectively integrated into modern systems and synthetic biology.

In trying to understand how cells work, there is a strong temptation, in the light of our accumulated molecular understanding, to pull them apart by perturbing individual components at DNA, RNA, or protein level. This "inside-out" strategy has been hugely informative—about components. Engineering offers an alternative "outside-in" strategy, in which a system is interrogated so as to reveal how it works. This offers, in principle, a more integrative approach.

A commonly used interrogation is to vary the frequency of stimulation. A system can be fully reconstructed if its complete frequency response is known. Even the high-frequency response reveals